on biological processes, such as gene expression. Two major challenges are that linker histones contain a long intrinsically disordered C-terminal domain (CTD) and determination of high-resolution structures of chromatin has not been possible due to the large size of the system and its dynamic nature.
We are taking advantage of the recently published 11-Å resolution electron microscopy maps of two chromatin fibers (Song et al., 2014), to construct a complete low-resolution model of chromatin. High-resolution crystal structures of nucleosome core particles are fitted to the electron density maps and the intervening segments of linker DNA are modeled based on a sampling protocol that utilizes new DNA optimization methods. Situs is used to score all models against the density maps. Linker histones are modeled on the densities extracted after accounting for the nucleosomes and linker DNA. The modeled paths of the DNA linkers and the sites of the nucleosomes provide spatial constraints for positioning the globular core and modeling the folding of the disordered CTD of the linker histone. The pathways of the CTD are selected from ensembles of conformations obtained using ab initio structure prediction tools like Rosetta. We are using the model in coarse-grained Monte Carlo simulations of long-range interactions along arrays of precisely positioned nucleosomes in the presence of linker histones.

2734-Pos Board B164
Matrix Constraints Regulate Transcription Dependent 3D Organization of Chromosomes
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Recent studies have revealed that cell geometric constraints regulate cytoskeleton as well as nuclear architecture, and gene expression. In addition, the spatial and temporal organization of chromosomes has been shown to modulate gene expression. However, the role of 3D organization of the nuclear architecture and chromosome assembly in facilitating this geometric-constraints regulated genome regulation is unclear. To address this, we used NIH 3T3 fibroblast cells cultured on fibronectin coated mircofabricated patterns, and combined fluorescence in situ hybridization (FISH) with confocal imaging. We found that alteration of cell geometry changed the radial position of individual chromosomes, as well as the relative position of specific chromosome pairs. Interestingly, such chromosome reorganization was accompanied with the enrichment of active RNA polymerase II (phospho S5CTD) in intermingling regions. Consistent with microarray results, we observed nuclear localization of serum response cofactors (MKL) in flattened nuclei, while that of inflammatory response transcription factors (p65) in spherical nuclei. Supporting this, super-resolution imaging of these factors showed spatial colocalization of SRF, MKL and pol2 in rectangular patterns, while colocalization of p65 and pol2 in circular patterns. More interestingly, binding-activatable localization microscopy (BALM) on open chromatin spreads revealed specific transcription dependent clustering of chromosomal contacts depending on nuclear morphology. In summary, our results reveal highly modular changes in 3D chromosome organization to facilitate co-clustering of genes and its co-regulation, depending on cell geometry.

2735-Pos Board B165
Mechanism and Function of Chromatin Positional Fluctuations in Interphase
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Chromatin structure and dynamics control all aspects of DNA biology yet are poorly understood. In interphase, time between two cell divisions, chromatin fills the cell nucleus in its minimally condensed polymeric state. Chromatin serves as substrate to a number of biological processes, e.g. gene expression and DNA replication, which require it to become locally restructured. These are energy-consuming processes giving rise to non-equilibrium dynamics. Chromatin dynamics has been traditionally studied by imaging of fluorescently labeled nuclear proteins and single DNA-sites, thus focusing only on a small number of tracer particles. Recently, we developed an approach, displacement correlation spectroscopy (DCS) based on time-resolved image correlation analysis, to map chromatin dynamics simultaneously across the whole nucleus in cultured human cells[1]. DCS revealed that chromatin movement was coherent across large regions (4-5μm) for several seconds. Regions of coherent motion extended beyond the boundaries of single-chromosome territories, suggesting elastic coupling of motion over length scales much larger than those of genes[1]. These large-scale, coupled motions were ATP-dependent and unidirectional for several seconds. Following these observations, we developed a hydrodynamic theory of active chromatin dynamics, using the two-fluid model and describing the content of cell nucleus as a chromatin solution, which is subject to both passive thermal fluctuations and active (ATP-consuming) scalar and vector events[2]. In this work we continue in our efforts to elucidate the mechanism and function of the chromatin dynamics in interphase by investigating the dynamic contribution of major nuclear motors such as DNA polymerase, RNA polymerase II, and topoisomerase II combining DCS and molecular perturbations.
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2736-Pos Board B166
Unique Mechanical Properties of Cell Nuclei Regulated by Chromatin
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Nuclear mechanics and structure could affect gene regulation and gene expression. Chromatin, a major component of cell nuclei, could play an important role in maintaining nuclear integrity and their mechanical properties. Previous studies on nuclear mechanical properties have focused largely on the role of the nuclear lamina, using techniques such as AFM and micropipette aspiration. In this work, we explicitly address the contributions of chromatin to nuclear rheology after isolation from the cell using a microfluidic optical stretcher. We find that isolated nuclei swell in volume under uni-axial stress and exhibit significant softening with increased nuclear size, which can be described by a filtration model for the nuclear membrane and a cortical chromatin model, respectively. In addition, changes to the state of chromatin condensation via histone modifications or chromatin remodeling processes (ATP, topoisomerase II) can strongly impact nuclear morphology and compliance. Moreover, isolated nuclear mechanics is also sensitive to ionic conditions: nuclei stiffen with increasing ionic strength of the buffer and exhibit a transition from stretch to contraction in the presence of multivalent ions (only). Finally, we find that in contrast to other studies suggesting a high refractive index of cell nuclei compared to the cytoplasm, the refractive index of isolated cell nuclei of a variety of cell types can be lower than the refractive index of the cells. The presented work establishes a quantitative link between nuclear mechanical properties and the compaction state of chromatin, which can be modulated by a change in nuclear volume, chromatin remodeling or electrochemical environment.

2737-Pos Board B167
Tracking Chromosome Conformation in Live Cells with CRISPR Imaging
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In each cell cycle, chromosomes go through dramatic large-scale structural changes, oscillating between being relatively open at interphase and highly compact at metaphase. However, little is known about how they change between the two extremes. Therefore it is desirable to monitor the long-term dynamics of chromosome structure in live cells. Here we use a modified CRISPR system to directly image specific genomic loci in hundreds of live cells with high temporal and spatial resolution. By following dozens of diffraction-limited fluorescence spots sparsely decorating a single chromosome, specific genomic elements can be localized with a precision of ~30 nm. We study how topological domains and long range interactions between chromosome loci are maintained or re-established through cell cycle. As CRISPR imaging allows us flexibility and specificity in imaging any genomic loci, the method developed here could be easily adapted to explore other systems where long-term live cell imaging is required.

2738-Pos Board B168
Conformational Sampling of Unmodified and Acetylated H3 Histone Tails on a Nucleosome by All-Atom Model Molecular Dynamics Simulations
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A nucleosome, 146 or 147 base-pair DNA wrapped around a histone octamer composed of two copies of each H3, H4, H2A, and H2B histone proteins, is a compact unit structure to store eukaryotic DNA into the cell nucleus. Although the X-ray conformations of the core region have been already determined, the conformations of the disordered histone terminal regions (histone tails) remain poorly understood. Recent experimental evidences suggest that chemical modifications on the histone tails regulate DNA functions, such as transcription, duplication, and splicing. To understand the regulation mechanism, it is necessary to elucidate difference between conformational states of unmodified and modified histone tails. Molecular dynamics (MD) simulations by generalized ensemble methods such as replica exchange MD and multicanonical MD are effective means to investigate the conformational ensemble